De-correlating the spatio-temporal signals of multi-field cortical activation patterns recorded by voltage sensitive dye imaging

Hess A [1,2,3] Hosokawa Y [1], Nasu M [1], Horikawa J [4], Taniguchi I [1,] Scheich H [2]

1 Medical Research Institute, Tokio Medical Dental University, Tokio, Japan
2 Leibniz Institute for Neurobiology, Brenneckestrasse 6, Magdeburg, Germany
3 Institute for Pharmacology and Toxicology, Fahrstrasse 17, Erlangen, Germany
3 Toyohashi University of Technology, Hibarigaoka 1-1, Toyohashi, Japan
email: hess@ifn-magdeburg.de

Abstract. Modern brain imaging techniques like voltage sensitive dye recording get highly resolved complex spatio-temporal datasets. For temporal classification of these complex 2D + t datasets we applied several statistical analysis approaches like principal component analysis and cluster analysis. These methods proved to be very helpful to characterise the cortical response patterns into known and unknown sub-regions. Whereas the cluster analysis showed the already known regions are parcellated even further, the principal component analysis and the statistical description of the time-course revealed as the main result a temporal order of the activation sequence of the different fields.

1 Introduction

The auditory system has to deal with time-varying stimuli. In particular the auditory cortex shows spatio-temporal complex response patterns due to different stimulus properties. Moreover, the auditory cortex is parcellated in a number of fields. Besides species specific differences up to seven different auditory cortex fields can be found [1,2,3]. The primary auditory cortex (AI) and at least one - the first - secondary (AII) field are tonotopically organized. These two areas are called core fields surrounded by the secondary fields in the belt area. For imaging we used the optical recording method of voltage sensitive dyes. This method is particularly well suited for such a study because it has a very high temporal resolution and enables simultaneous recording of multiple sites. By using a new multi-field recording scheme we could achieve highly resolved recordings in time and space of the complete guinea pig auditory cortex in response to different stimuli. However, the functional specialization of these fields remains unclear. In this study we tried to establish an analytical framework for characterizing these 3D datasets of the different fields according to their temporal response properties (third dimension) by several image processing strategies. Beside descriptive statistics we tried to group time courses of the pixels using explorative statistical methods.

2 Recording Methods

Nembutal anesthetized animals were artificially intubated and positioned in a stereotactic frame. A hole was drilled into the bone above the auditory cortex and the auditory cortex exposed. Afterwards, the dura and the arachnoid membrane were removed and the cortex stained for 90 min with a voltage-sensitive dye (RH795; 0.2 mg/ml dissolved in saline, Molecular Probes, USA). The recording was performed in a sound-proof chamber and with artificial respiration (60 % O_2) after injection of Mioblock and supplementary doses of Nembutal. The animal was placed under a microscope and the auditory cortex illuminated with light between 480 and 580 nm. The emitted fluorescent light was filtered at 620 nm and recorded with a 144 (12x12) channel photodiode array, amplified by x2000, filtered between 1 Hz and 10 kHz and sampled at a frame rate of 0.576 ms per frame. A complete view of the whole auditory cortex was reconstructed by means of a computational alignment procedure out of several single recording positions, whose individual sizes were restricted by certain optical properties. At each recording position five trials were averaged for each stimulus frequency (4, 8, 12, 16 kHz). The sound stimuli used had a 10 ms rise and fall time and a duration of 50 ms. The stimuli were presented at 75 dB SPL.

3 Image analysis

The time courses for each pixel were analysed in three different ways. We calculated a first order statistical description of the time course of the changing optical signal (see Fig. 1), a principal component analysis, and a cluster analysis of the timecourse of each pixel. The following first order parameters were introduced to characterize the time courses of the voltage-sensitive dye signals: Onset and offset are in our case defined as the first (onset) resp. last (offset) time point where the intensity is more than one times larger than the mean plus one time the standard deviation of the total time course. The peak is defined as the overall maximal value of the time course. Peak cutting start and end values are obtained at a given percentage level (usually 60 %) of the peak value. Additionally, we introduced the principal component analysis (PCA) to find time-response-functions, which are orthogonal and therefore independent of each other. If the back projections of such components are adjacent and located within one field, this would proof, that this field shows a homogeneous temporal response property. Due to the fact, that the different components are also ranked by their contribution strength to the original signal, one also gets information about the dominance of certain temporal response properties.

4 Results

Using the first order statistical parameters we were able to separate the different auditory fields mainly by their latency and their duration [3].

The principal component analysis was able to differentiate between the different auditory fields especially between the primary and secondary auditory fields.

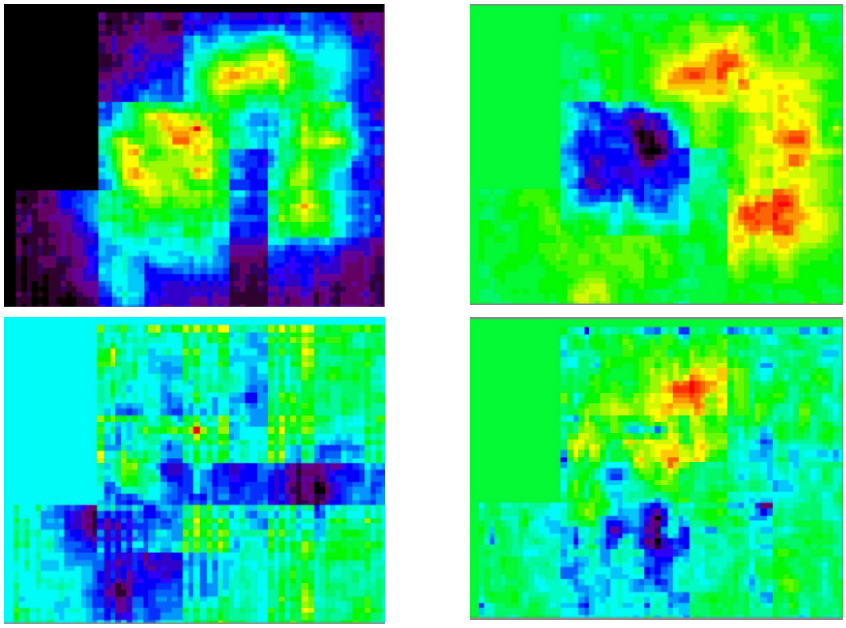

Fig. 2. The back-projecting of the first four (C1-C4) principal components of 8 kHz auditory stimulation. Note, the dark blue areas in C2 are the primary, the core fields. Images are individually scaled!

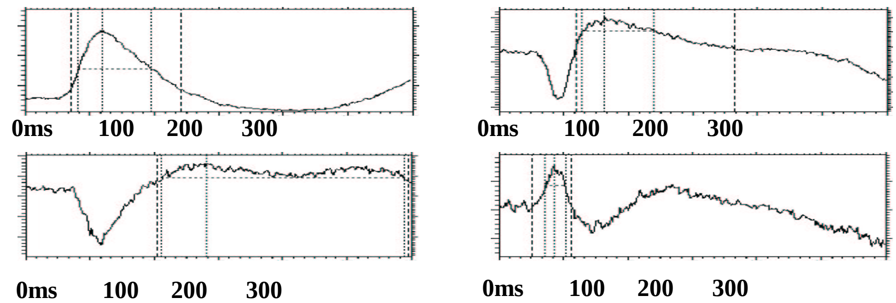

Fig. 3. The time course of the back-projecting of the first four (C1-C4) principal components of 8 kHz auditory stimulation.

In detail following interesting neurobiological results were obtained:

- The core fields are activated first and afterwards the activity spread via three different pathways to the belt region (dorsal, caudal and ventral).
- The core fields AI and AII show the highest variance in the data set, which strongly determines the first component.
- The time course of activity is nearly the same in AI and AII and it is fast.
- The second component is mainly determined by the secondary - belt areas.
- The variance of component three is small in the ventral and caudo-medial fields. This indicates that the variance of these fields is already explained by component 1 and component 2.
- Component four shows small spots of high variance in AI, AII and sometimes D. This very early and transient response of component four suggests that this component reflects the thalamic inputs into the auditory cortex.
- Lower stimulation frequencies need more components to explain the same level of variance in the datasets.

The cluster analysis showed the parcellation of similar time courses into clusters which are smaller than the already described auditory fields.

5 Discussion

In summary supported by all analysis strategies we found, that the belt fields were activated later than the core fields and that the activity spread from core fields to belt fields via three independent different pathways: to the dorsal, caudal and ventral direction. Most prominently the different belt fields could be separated by their activation duration. Moreover, the principal component analysis was able to separate several temporal response components. The first of these components could represent the input into the cortex because it had the shortest latency and only appeared in the primary fields, which do have a direct thalamic input into the cortex. The cluster analysis on the other hand, showed that there is a diverse parcellation of the auditory cortex into areas showing comparable response characteristics. These clusters are smaller than the already described fields and have to be further investigated. By computing a Spearman rank order statistic, we were able to get from this analysis framework a specific response pattern for each field.

6 References

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